The application of Next Generation Sequencing MODY Gene Panel in Greek Patients

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Introduction: Maturity Onset Diabetes of the Young (MODY) constitutes a genetically and clinically heterogeneous type of Monogenic Diabetes (MD). It is characterized by autosomal dominant inheritance, early onset of diabetes (≤25 years), defect in the β-cell insulin secretion, positive family history of diabetes, absence of diabetic ketoacidosis, auto-antibodies (ICA, anti-GAD or IAA), insulin resistance. Patients usually have normal Body Mass Index [1]. To date, 14 different MODY subtypes have been reported each one with a distinct genetic etiology [2]. The most common MODY subtypes are MODY1-HNF4A, MODY2-GCK, MODY3-HNF1A and MODYS-HNF1B.

Objective: To identify the molecular defect of 49 MODY patients employing the methodology of Next Generation Sequencing (NGS) Targeted Gene Panel.

Patients and Methods: We studied 49 patients who met MODY criteria (Table 1). A panel of seven MODY genes (GCK, HNF1A, HNF4A, HNF1B, INS, ABCB8 and KCNJ11) sized 29.45kb with 98.87% in silico coverage was designed by the Thermo Fisher Scientific Ion AmpliSeq Designer platform (version 5.6) according to hg19. NGS was performed on the Ion Torrent Personal Genome Machine (PGM) platform (Thermo Fisher Scientific, Waltham, MA, USA) using the Ion PGM™ Hi-Q View Sequencing Kit and ion 314™ chip v2. Bioinformatic tools were used to test the pathogenicity of the new variants detected. The pathogenic variants detected in the patients and the parent with the MODY phenotype when available, were also tested by Sanger sequencing.

Results: Thirteen pathogenic variants were identified in 12 of the 49 MODY patients tested (24%). The variants were: 2 nonsense, 10 missense and 1 splice site (Table 2). Four novel pathogenic variants were detected in the GCK (p.Cys371X), HNF1A (p.Asn402Tyr), HNF4A (p.Glu285Lys) and ABCB8 (p.Met1513Thr) genes. Four patients (32%) were found to be heterozygotes for GCK variants, two (16%) for HNF1A variants, one (8%) for HNF4A variant, one (8%) for HNF1B variant and five (42%) for ABCB8 variants. Interestingly, one patient was found to carry two different gene variants, one of the GCK gene (p.Tyr61X) and one of the ABCB8 gene (p.Leu135Val). The combination of these two variants may lead to a reduced response of the β-cells at high glucose levels and a reduced insulin secretion. Two patients carried de novo pathogenic variants of the GCK gene (p.Ala259Thr) and HNF4A gene (p.Glu285Lys), respectively. No pathogenic variants were detected in the KCNJ11 and INS genes.

Conclusions: The application of NGS targeted gene panel of 7 MODY genes offered genetic diagnosis in 24% of the patients tested and revealed four novel gene pathogenic variants and a digenic inheritance case. The majority (42%) of the detected pathogenic variants were in the ABCB8 gene, indicating that MODY12 cases are probably more common than previously considered. Although a large number of MODY patients remain without the exact MODY type identification, the application of NGS methodology in diagnosis provides rapid results, is cost effective compared to Sanger sequencing and increases diagnostic accuracy. It is probable that the employment of a panel with more genes associated with monogenic diabetes will allow the molecular defect identification in more patients.


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